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SOIL MYCOFLORA OF BLACK PEPPER RHIZOSPHERE IN THE PHILIPPINES AND THEIR *IN VITRO* ANTAGONISM AGAINST *Phytophthora capsici* L.

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ABSTRACT

Foot rot disease of black pepper caused by *Phytophthora capsici* had been reported in Batangas and Laguna, Philippines. The plant was recovered following the application of crop residue (organic substrate) and intercropping with other crops. This study was aimed to isolate, identify, and determine the soil mycoflora from the rhizosphere of black pepper grown on various cropping patterns in Batangas and Laguna. Antagonistic activity of mycoflora isolates was tested against *P. capsici* using dual culture technique. The result showed that 149 colonies of soil mycoflora isolated were belonging to 14 genera; three of them, i.e. *Penicillium*, *Paecilomyces* and *Aspergillus*, were the most dominant. All of the mycoflora isolates were able to inhibit the growth of the pathogen. Eighteen of them were the most promising antagonists, based on their inhibition growth of more than 60%. It is suggested that antagonistic mechanism of *Mucor* isolate (1001), *Trichoderma* (125, 170, 171, 179, 180, 181), *Gliocladium* (109), *Cunninghamella* (165, 168), *Mortierella* (177), and *Aspergillus* (106) was space competitor (competition for nutrient) since they rapidly overgrew the pathogen. *Aspergillus* (67, 79, 81, 83, 108, and 202) isolates inhibited the pathogen apparently by producing antibiotic, whereas *Trichoderma* (125, 170, 171, 179, 180, and 181) isolates were able to penetrate the hyphae of the pathogen. The organic matter percentage in the soil was significantly correlated with the number of antagonistic mycoflora in rhizosphere (R^2 = 0.1094), but the cropping pattern was negatively correlated. This study suggests that organic matter increased antagonistic mycoflora in black pepper rhizosphere, which will reduce severity of the disease.

[*Keywords*: *Piper nigrum*, fungi, microbial flora, rhizosphere, microbial pesticides, *Phytophthora capsici*]

INTRODUCTION

Phytophthora capsici Leon is a soilborne pathogen and the causal agent of foot rot disease of black pepper *(Piper nigrum* L.*)* (Anith *et al.* 2002). The disease has been found in major black pepper plantations in the Philippines such as Provinces of Batangas, Laguna, Quezon, Basilan, Zamboanga, and Davao (PCARRD 1990). In 2001, the disease caused a serious damage on a black pepper plantation at Barangay Quezon in Lipa, Batangas Province. The disease disappeared following adoption of growing black pepper intercropped between rows of coffee, cacao, and lanzones or other fruit trees, and covering plants with crop residues (PCARRD 1990; Mr. Celso, pers. comm.).

Rhizosphere contains lots of organic substrates which harbor a high count of microorganisms, especially fungi. Species richness is usually high in the rhizosphere and root-free soil, but distinctly low in the rhizoplane. It has been estimated that one gram of surface soil contains 50,000 to a million fungi (Wahid *et al*. 1997). The loss of organic materials from roots provides the energy for the development of active fungal population in the rhizosphere around the root. Generally, saproptrophs or biotrophs grow in the rhizosphere in response to this carbon loss, but plant pathogens may also develop and infect a susceptible host, resulting in disease (Whipps 2001).

Fungal species and their population in the soil vary depending on many factors such as type of soil, vegetation, temperature, organic matter, and moisture content. Dissanayake and Hoy (1999) indicated that soil amendment with organic materials may provide a sustainable, effective biological disease control option for soilborne plant diseases. Microbial activity and mineral nutrient availability are correlated with soilborne disease incidence and severity. Anandaraj (2000) reported that in the rhizosphere of black pepper, several antagonistic microorganisms belonging to *Trichoderma* and *Gliocladium* occur. Mechanism of action involved in fungal interaction in soil is assessed with particular emphasis on antibiosis, competition, parasitism, and induced resistance (Whipps 2001).

Studies on the biological control of the foot rot disease on black pepper are mainly focused on identifying microorganisms from rhizosphere of black pepper that can effectively suppress foot rot disease. Among the fungal antagonists, species of *Trichoderma* are the most potential agent for bio-control. This fungus inhibited the production of sporangia and lysed the mycelium and zoospore of *P. capsici* (Manohara *et al.* 1992). In greenhouse experiment, *T. harzianum* (TSM) reduced 50% of incidence of *P. capsici* at 4 months after planting (Manohara and Kasim 1996). The following studies were conducted to identify the soil fungi of the rhizosphere of black pepper grown under various cropping patterns in Batangas and Laguna, and to determine their antagonistic activities against *P. capsici in vitro*.

MATERIALS AND METHODS

Soil samples were collected from areas grown with black pepper alone and those cultivated with black pepper and other crops such as ubi *(Dioscorea alata* L.*)*, lanzones *(Lansium domesticum* Corr.*)*, and coffee *(Coffea* spp.*)* in Laguna and Batangas, Philippines. The studies were conducted at the Plant Pathology Laboratory, University of the Philippines Los Baños (UPLB) in 2002-2003.

One kilogram soil samples (included plant debris) were collected with a soil auger at a depth of 10-25 cm from the rhizosphere of black pepper plantations. The samples were placed in clean plastic bags. Five soil samples taken (four at the corner and one at the center) from each study site were mixed thoroughly into one composite sample. Ten-gram subsamples of soil from each composite sample were processed for isolation of soil mycoflora. In cases where processing of the samples could not be done on time, the samples were refrigerated (4^{o} C) for not more than 2 weeks (Sinaga and Quimio 1987).

Soil Analysis

Soil samples from each area studied were analyzed for pH, organic matter, and content of total N, P and K by sending them to the Analytical Services Laboratory, Department of Soil Science, UPLB.

Isolation of Mycoflora from Soil

The mycoflora of the soils was isolated following the soil dilution-plating technique of Johnson *et al.* (1960). A. sample of 10 g soil was placed in a graduated cylinder, added with sterilized distilled water to make a total of 100 ml. The suspension was stirred, poured into sterile 250 ml Erlenmeyer flask and shaken thoroughly for 30 minutes. One milliliter of this suspension was pipetted aseptically and dispensed in dilution test tubes with 9 ml of sterilized distilled water. Series of soil dilutions of 1:10, 1:100, 1:1,000 and 1:10,000 were prepared. One milliliter of the desired dilution (10^{-3} and 10^{-4}) was transferred aseptically into a sterile petri dish and with 10-12 ml of melted potato dextrose agar (PDA with one drop of 20% lactic acid) (Sinaga and Quimio 1987). The dish was rotated by hand in a broad and slow swirling motion to disperse the soil suspension. Three petri dishes were provided for each dilution. Plates were incubated at room temperature for 5 days. After incubation, a small portion of mycelium from each fungal colony was transferred into PDA slants.

Pure cultures of isolates were grown on standard media and few selective media, and identified using the fungal keys provided by Domsch *et al*. (1980), Sinaga and Quimio (1987), Singh *et al.* (1991), Quimio and Hanlin (1999), and Quimio (2001). The soil fungi isolated were deposited at the Mycology Laboratory of the Department of Plant Pathology, UPLB.

The abundance (%) of soil mycoflora on various crop combinations was determined using this formula:

$$\text{Abudance} = \frac{\text{the number of fungal colonies of genus A per study site}}{\text{total number of fungal colonies per study site}} \times 100\%$$

Isolation of *P. capsici*

Phytophthora capsici was isolated from diseased stem and roots of black pepper collected from a field in Cotabato, Mindanao following the technique described by Singleton *et al.* (1992). Pathogenicity of the *P. capsici* isolate was tested at the greenhouse of the Department of Plant Pathology, UPLB.

Screening of Isolates Against *P. capsici*

All of the mycoflora isolated were individually tested for their antagonistic property against *P. capsici* using the dual culture technique. Small same sized agar pieces of *P. capsici* and other fungi (4-day old) were placed on plates of PDA. The distance between *P. capsici* and the mycoflora was about 5 cm. Plates

were incubated at room temperature for 5 days. Three plates were provided per isolate. Plates inoculated with *P. capsici* alone served as control.

Clear zone of inhibition (CZI) was also determined by measuring the clearance between the margins of the *P. capsici* and mycoflora. Radial growth of both *P. capsici* and mycoflora were measured 5 days after inoculation. Percent inhibition of radial growth (PIRG) was measured using the formula:

$$PIRG = \frac{R1 - R2}{R1} \times 100$$

where, R1 is the diameter (cm) of colony growth of pathogen in control; R2 is the diameter (cm) of the pathogen in antagonist-tested plate. Descriptive assessment of the antagonistic activity was scaled as follows (Soytong 1988):

++++ = very high antagonistic activity (>75 PIRG)
+++ = high antagonistic activity (61-75 PIRG)
++ = moderate antagonistic activity (51-60 PIRG)
+ = low antagonistic activity (<50 PIRG)
- = no activity

Microscopic examinations of the mycelia within overlapping areas were done after 4 days using scanning electron microscopy (SEM) at the Microscopy Service Laboratory (EMSL) BIOTECH, UPLB.

Data Analysis

The data were analysed using analysis of variance in completely randomized design using general linear procedure of SAS and SPSS version 10.0 software. Least significant difference was used in comparing the treatment means. Regression correlation analysis was done between percent organic matter content of soil from different study sites with the total number of fungal colonies isolated per gram soil and with that of the number of colonies of promising antagonist fungal species.

RESULTS AND DISCUSSION

Soil Mycoflora

One hundred and forty nine colonies of mycoflora were isolated from soil samples taken from six study sites (Table 1). Significant variation in total number of fungal colonies isolated was observed in soil samples from various study sites. Sto. Tomas, Batangas (planted with black pepper alone) showed the highest number of fungal colonies isolated (42) with nine promising antagonists, followed by Cuenca, Batangas (black pepper grown with ubi) with 31 fungal colonies isolated with four promising antagonists. The lowest number of fungal colonies isolated was found in Los Baños, Laguna site, with the soil coming from the rhizosphere of black pepper alone, with six fungal colonies isolated, two of which are promising antagonists. These results showed that factors such as organic matter in soil have an effect on the population of fungi and the presence of antagonists in the soil. Soils with high levels of organic matter have a more complex and active mycoflora associated with their ability to suppress the activity of the root rot pathogen, *Phytophthora cinnamomi* Rands (Hoitink and Boehm 1999).

The primary important factors for the action of antagonists during the competition for nutrients in the soil are competition, crop choice, and organic amendment. Fungicide application may affect biocontrol of some soil pathogens. Successive cropping may be necessary to build up populations of the antagonists. According to Dissanayake and Hoy

Table 1. Total number of fungal colonies isolated from 10-g soil samples from Batangas and Laguna, Philippines, the number of promising antagonist isolates, and chemical analysis of soil.

Study site	Cropping pattern[1]	Total number of fungal colonies isolated	Number of promising antagonists	pH	Organic matter (%)	N (%)	P (ppm)	K (cmol kg^{-1} soil)
Sto. Tomas, Batangas	BP alone	42	9	5.6	4.98	0.25	39	3.1
Cuenca, Batangas	BP grown with ubi	31	4	6.3	2.08	0.14	93	4.5
Lipa, Batangas	BP grown with lanzones	26	3	4.9	2.86	0.16	14	2.0
Lipa, Batangas	BP alone	23	0	5.2	1.83	0.11	17	3.9
Sto. Tomas, Batangas	BP grown with coffee	21	0	6.1	3.09	0.18	2	1.9
Los Baños, Laguna	BP alone	6	2	5.4	2.20	0.14	25	2.6
Total		149	18					

[1]BP = black pepper.

(1999), increases in plant growth resulting from soil amendments with different organic materials were documented for various crops and attributed to improvements in nutritional and physical soil properties and microbial interactions, including soil-borne disease suppression.

Regression analysis showed significant correlation between the percentage of soil organic matter and the number of fungi isolated, and also between the percentage of soil organic matter and the number of promising antagonists present in the soil of study sites at the 5% level (Fig. 1). However, the number of fungi and promising antagonists were negatively correlated with cropping pattern, pH, N, P and K content in the soil (data not shown). Coefficient determination (R^2) of the linear regression between organic matter (%) and number of fungi was 0.1094, and between organic matter (%) and number of promising antagonist isolated was 0.1253. In this case, the model can only explain 10.94% and 12.53% effect of organic matter on variation in the number of fungal colonies and promising antagonist isolated in the soil, respectively. It means that other factors such as type of soil, temperature, moisture content, and competition between organism present in the soil may affect the number of fungi and promising antagonist isolated. These factors need to be studied further.

The nonsignificant intercept in the simple regression at 5% level means that the absence of organic matter (%) did not affect the presence of fungi or promising antagonist in the soil. The population of antagonistic mycoflora in rhizosphere would affect the population of pathogen *(P. capsici)* in the soil. Hoitink and Boehm (1999) stated that the decomposition level of organic matter critically affects populations of antagonistic microorganisms and hence the degree of the disease control achieved. The competitive saprophytic ability of *P. capsici* was very low and addition of organic matter to the soil containing *P. capsici* promotes the growth of saprophytes and reduces the population of *P. capsici* (Anandaraj 2000).

Genera of Fungi Isolated from Soil and Percentage Abundance of Isolates under Various Cropping Patterns

There were 14 genera of fungi from soil samples collected from the rhizosphere of black pepper under various cropping patterns in Batangas and Laguna (Table 2). The genera *Penicillium* and *Paecilomyces* have the most number of isolates among all the

Table 2. List of genera with the number of isolates of soil mycoflora from soil samples collected from Batangas and Laguna, Philippines.

Group	Genera	Number of isolates
Zygomycetes	*Cunninghamella*	7
	Mortierella	7
	Mucor	1
Hyphomycetes	*Acremonium*	3
	Aspergillus	27
	Botrytis	1
	Geotrichum	1
	Gliocladium	3
	Paecilomyces	40
	Penicillium	41
	Trichoderma	6
	Verticillium	7
Coelomycetes	*Phoma*	1
Ascomycetes	*Nannizzia*	4
Total		149

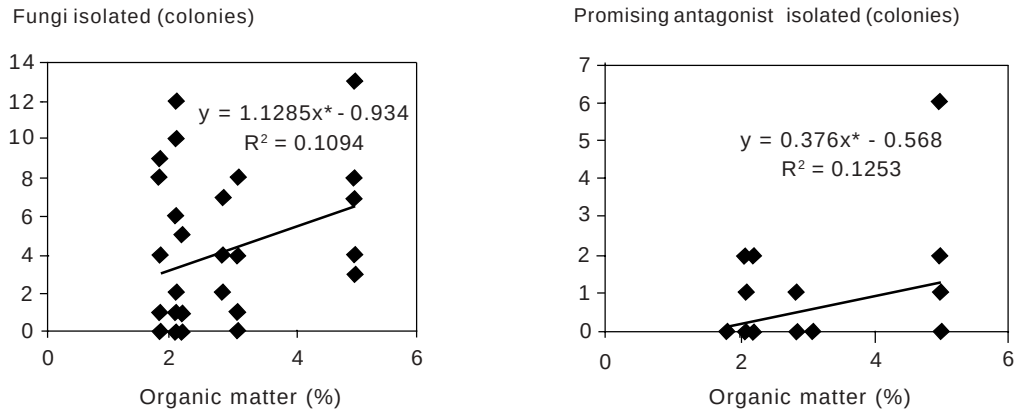

Fig. 1. Correlation of organic matter and the number of fungi isolated (left) and promising antagonists isolated (right) from soil samples collected from Batangas and Laguna, Philippines as determined by regression analysis ($P < 0.05$).

genera of class Hyphomycetes with 41 and 40 isolates, respectively, followed by genus *Aspergillus* with 27 isolates. On the other hand, Zygomycetes and Ascomycetes were represented by 15 and 4 isolates respectively, then Coelomycetes with one isolate. Warcup (1970) reported that the most common fungi isolated using dilution plates from wheat field soil are Fungi Imperfecti, Mycelia Sterilia, Phycomycetes, and Ascomycetes. According to Wahid *et al.* (1997), the genera *Aspergillus* and *Penicillium* are the richest amongst all the genera of class Hyphomycetes found in soils of tomato field. It was reported by Domsch *et al.* (1980) that *Aspergillus niger* was found in soil with pH range of 4-8. There was an increase in relative numbers after soil fumigation, after manuring and NPK fertilization.

Forty two species of field and storage fungi were isolated in black pepper and white pepper. *Aspergillus flavus* and *A. niger* were encountered most frequently, prevalence being greater in the black pepper (Freire *et al.* 2000). Domsch *et al*. (1980) also reported that *Cunninghamella elegans* is particularly frequent from cultivated soils, but it has also been reported from uncultivated soils, grassland, a vineyard with a high copper content, and rice fields. There are also numerous reports from forest soils. *C. elegans* grows particularly well in moist habitats. It has also been isolated from litter of various deciduous trees, *Eugenia heyneana*, and *Eucalyptus maculata*. Its occurrence on banana roots has been observed repeatedly, but it is also known from roots of oat, barley, sugar cane, *Pinus taeda* and the rhizosphere of groundnuts, tobacco, *Rauwolfia canescens*, *Aristida coerulescens*, papaya plants and sugar cane. Lewis and Papavizas (1985) mentioned that *Trichoderma* in natural soil requires substrates as source of nutrients to enhance growth, survival, and competitiveness. *Trichoderma* occurs relatively frequently in the surface (litter) layer of soils and generally in slightly acid habitats.

Table 3 shows the abundance of fungi genera isolated on various cropping patterns in black pepper rhizosphere plantations. No diseases were observed at all of study site. Statistical analysis showed a negative correlation between genera and cropping pattern and or study site.

Isolation and Identification of *P. capsici*

Phytophthora capsici was isolated from diseased black pepper collected from Cotabato, Mindanao, Philippines. The species was confirmed using the Tabular Key to Species of *Phytophthora* (Stamps *et al.* 1990), and based on the findings of Rellon (1990) and Tangonan (1999). It is characterized by having a coenocytic mycelium of fairly coarse hypha, 5-7 µm wide (x = 5.9 µm, n = 10). The sporangiophore is narrow, sometimes widening slightly at the base of the sporangium, branching irregularly, and forming sporangium at each end. The sporangia are often irregular, spherical or ovoid, 45-75 µm long (x = 57 µm, n = 10), often distorted and with more than one apex, papillate. The isolate was pathogenic following the inoculation on black pepper plant (one-month old).

Screening of Isolates Against *P. capsici*

All isolates were able to inhibit the growth of pathogen on PDA plates *in vitro* with percentage of inhibition of radial growth between 4.88% and 72.73%. The isolates were regarded as promising antagonists when there was more than 60% inhibition of radial growth of the pathogen as a result of their activities. Eighteen promising antagonist isolates were found among 149 isolates collected from rhizosphere of black pepper under various cropping patterns.

Table 4 shows the antagonistic activity of promising isolates on PDA after 5 days of incubation. There are differences in inhibition of radial growth of *P. capsici*. *Mucor* (isolate no. 101) showed the highest antagonistic activity with the highest PIRG of 75.55%, followed by *Aspergillus* (106) 71.08%, *Aspergillus* (108) 67.06%, *Trichoderma* (170) 66.88%, *Aspergillus* (202) 66.87%, *Gliocladium* (109) 66.36%, and *Trichoderma* (179) 66.23%. Antagonistic activities of *Mucor* (101) were significantly different with all of promising isolates tested. According to Shearer (1995), *Mucor hiemalis* was the better competitor in agricultural soil because it is in the fungal order Mucorales, the taxa of which are considered to be r-selected and poor competitors. The success of *M. hiemalis* may be a result of its rapid growth rate, which may have enabled the fungus to monopolize resources faster.

All *Aspergillus* isolates produced antibiotic compound shown by clear inhibition zone, except *Aspergillus* (106) isolate (Table 4). *Aspergillus* (79) showed the highest clear zone (6.67 mm), followed by *Aspergillus* (81) and *Aspergillus* (202) with 4.67 mm and 2.78 mm, respectively. Antagonistic interactions of fungi can be mediated either by direct contact or at a distance. The former involves direct physical contact between the two organisms, while the latter refers to those instances in which one fungus releases materials such as antibiotics and lytic enzymes into

Table 3. Abundance of fungal genera from rhizosphere of black pepper under various cropping patterns in Batangas and Laguna, Philippines.

Study site	Cropping pattern	Soil mycoflora and their isolate numbers[1]	Abundance (%)
Sto. Tomas, Batangas	Black pepper alone	*Aspergillus* (158, 159, 160, 166, 176, 186, 187, 188, 201, 202)	23.81
		Cunninghamella (163, 164, 165, 167, 168, 183, 185)	16.67
		Mortierella (169, 172, 174, 175, 177, 178, 184)	16.67
		Penicillium (189, 190, 191, 192, 193, 194, 195)	16.67
		Trichoderma (170, 171, 179, 180, 181)	11.90
		Paecilomyces (157, 161, 162, 173, 182)	11.90
		Geotrichum (200)	2.38
Cuenca, Batangas	Black pepper grown with ubi	*Aspergillus* (67, 70, 75, 79, 81, 83, 84, 88, 89, 90, 91)	35.48
		Verticillium (68, 69, 80, 82, 86, 92, 93)	22.58
		Penicillium (64, 71, 72, 73, 74, 85, 87)	22.58
		Paecilomyces (66, 76, 94, 95)	12.90
		Nannizzia (77, 78)	6.45
Lipa, Batangas	Black pepper grown with lanzones	*Penicillium* (96, 97, 98, 100, 103, 111, 112, 114, 115, 117, 121, 122, 123)	50.00
		Paecilomyces (99, 102, 105, 110, 116, 120)	23.08
		Aspergillus (104, 106, 118)	11.54
		Gliocladium (109, 113)	7.69
		Botrytis (119)	3.85
		Mucor (101)	3.85
Lipa, Batangas	Black pepper alone	*Paecilomyces* (204, 206, 208, 209, 210, 211, 213, 214, 215, 216, 217, 218, 219, 222, 223, 225, 227)	73.91
		Penicillium (207, 212, 220, 221, 224, 228)	26.09
Sto. Tomas, Batangas	Black pepper grown with coffee	*Paecilomyces* (130, 135, 139, 140, 141, 144, 150, 156)	38.10
		Penicillium (128, 131, 134, 136, 137, 151, 154)	33.33
		Acremonium (145, 146, 148)	14.29
		Nannizzia (133, 153)	9.52
		Aspergillus (129)	4.76
Los Baños, Laguna	Black pepper alone	*Aspergillus* (108, 126)	33.33
		Gliocladium (107)	16.67
		Penicillium (124)	16.67
		Trichoderma (125)	16.67
		Phoma (127)	16.67

[1]List of the isolates were stored in Department of Mycology, UPLB.

the environment that induce a negative effect on the other. *Aspergillus* species provide several antifungal metabolites such as nominine, aflavinines, paspalinine, and aspernomine (Gloer 1995). According to Domsch *et al.* (1980), *Mucor racemosus* produces a substance with antibiotic activity against various test bacteria. *Mortierella alpina* has antagonistic activity against *Rhizoctonia solani* and *Gaeumannomyces graminis*. The culture filtrate of *Penicillium canescens* was found to inhibit bacteria and fungi. *P. claviforme* was the most efficient antagonist of 118 fungi and bacteria tested against *Heterobasidion annosum*. *P. janthinellum* is antagonistic towards various bacteria while fungi *Chalara elegans*, *R. solani*, *Gaeumannomyces*, *Pythium ultimum*, on human pathogens. *P. jensenii* reported to inhibit *Staphylococcus aureus*, *Candida albicans*, *Botrytis aclada*, and *R. solani*. *Aspergillus erythrocephalus*

Table 4. Promising antagonist isolates with their comparative extent of inhibition against *Phytophthora capsici.*

Isolate number	Genus	PIRG[1]	Clear zone (mm)	Antagonistic activity[2]
101	*Mucor*	75.55a	0.00[3]	++++
106	*Aspergillus*	71.08ab	0.00	+++
108	*Aspergillus*	67.06bc	1.11	+++
170	*Trichoderma*	66.88bc	0.00	+++
202	*Aspergillus*	66.87bc	2.78	+++
109	*Gliocladium*	66.36bc	0.00	+++
179	*Trichoderma*	66.23bc	0.00	+++
67	*Aspergillus*	65.99bcd	1.89	+++
171	*Trichoderma*	65.96bcd	0.00	+++
79	*Aspergillus*	65.59bcd	6.67	+++
180	*Trichoderma*	65.24bcd	0.00	+++
168	*Cunninghamella*	64.62bcd	0.00	+++
181	*Trichoderma*	64.54bcd	0.00	+++
83	*Aspergillus*	63.90bcd	1.78	+++
81	*Aspergillus*	63.04cd	4.67	+++
177	*Mortierella*	61.41cd	0.00	+++
165	*Cunninghamella*	60.62cd	0.00	+++
125	*Trichoderma*	58.74d	0.00	++
Control	*Phytophthora capsici*	0.00e		-

[1]PIRG = percent inhibition of radial growth.

[2]Antagonistic activity: ++++ = very high antagonistic activity (>75 PIRG); +++ = high antagonistic activity (61-75 PIRG); ++ = moderate antagonistic activity (51-60 PIRG), + = low antagonistic activity (<50 PIRG), - = no activity.

[3]no clear zone = overgrown.

Means with the same letter in the same column are not significantly different at 5% level, based on LSD.

has antibiotic activity against *Pythium irregulare*, *Escherichia coli,* and *S. aureus*.

Mechanisms of Activity

The ability of *Mucor* (isolate no. 101), *Trichoderma* (125, 170, 171, 179, 180, 181), *Gliocladium* (109), *Cunninghamella* (165, 168), *Mortierella* (177), and *Aspergillus* (106) to grow rapidly is a probable mechanism by which these organisms were able to control the growth of the pathogen. The effective antagonists grew at a very fast rate outpacing the growth of the pathogens. The antagonists completely overgrew the pathogen and covered the entire medium surface after 5 days of incubation. Their interaction showed inhibition of growth of the pathogen by competition for nutrient.

Scanning electron microscopy (SEM) of agar blocks taken from the zone of contact showed coiling of hyphae of *Trichoderma* (179), *Gliocladium* (109), and *Mucor* (101) on the hyphae of *P. capsici* (Fig. 2), followed by hypha penetration through a haustorium. Haustorium penetration resulted in hyphal mating of *Trichoderma* (179) and the pathogen (Fig. 3). Their interaction showed inhibition of growth of the pathogen by parasitism. In studies on the antagonistic properties of *T. harzianum*, it was observed that hypha coils around or invades the hyphae of several tested fungi. Some isolates have been found to be antagonistic to *Candida albicans*, *R. solani*, *Armillaria mellea,* and *Lentinus edodes* (Domsch *et al.* 1980). Other possible mechanisms of biocontrol are by mycoparasitism (Jeffries 1995) and antibiosis (Fravel and Keinath 1991).

According to Cumagun and Ilag (1997), parasitism was revealed by coiling of *T. harzianum* around the hyphae of the pathogen *(R. solani)* causing distortion, broken segments, and vacuolation. The antagonist produced lytic enzymes, which degraded the hyphal walls of the pathogen, which would later result in the lysis of its cells (Cuevas *et al.* 1995). Lysis of hyphae or spore stages is a frequently cited mechanism of antagonism used by fungi against soilborne plant pathogens growing in highly organic soils (Malajczuk 1983). *Trichoderma* produces a

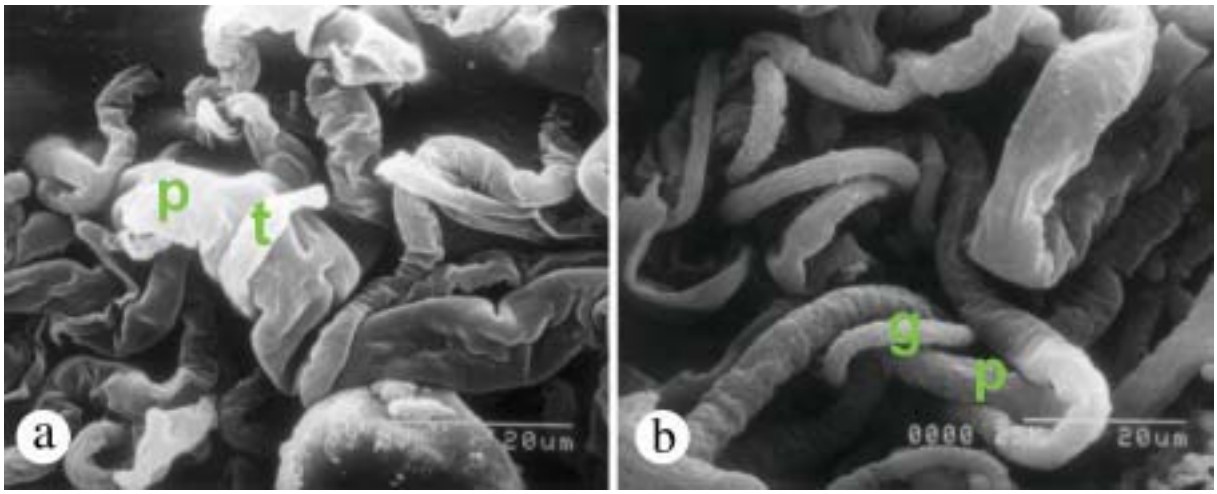

Fig. 2. Scanning electron microscopy taken at the point of contact between the isolates of antagonists and *Phytophthora capsici* (p); a = hyphae of *Trichoderma* (isolate no.179) (t) coiling the hypha of *P. capsici* (2000x); b = hyphae of *Gliocladium* (isolate no.109) (g) coiling the hypha of *P. capsici* (2000x).

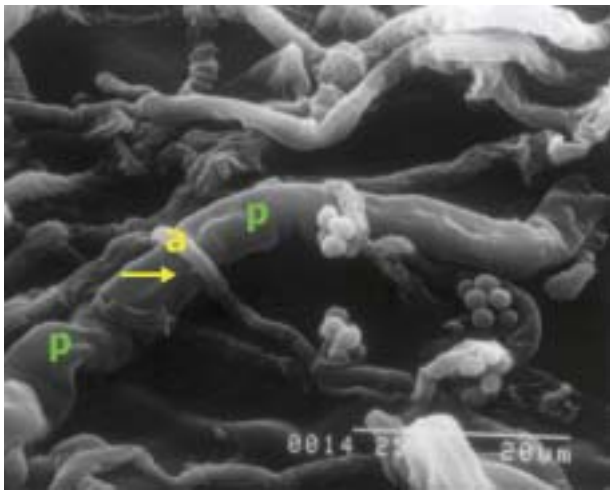

Fig. 3. Scanning electron microscopy of *Trichoderma* (isolate no. 179) hyphae (a) penetrating into the hyphae of *Phytophthora capsici* (p) through a haustorium (arrow) (2000x).

complex array of chitinolytic and glucanolytic enzymes which may be involved in the mycoparasitic activity (Harman *et al*. 1993). Antifungal compounds produced from *T. harzianum* and *T. longibrachiatum* are 2-phenyl-ethanol, tyrosol, 6-n-pentyl-α-pyrone and sorbicillin (Tarus *et al*. 2003).

Hyphae of *P. capsici* ceased to grow upon contact with the hyphae of *Aspergillus* (67, 79, 81, 83, 108, 202) isolates. The antagonist apparently produced some substance (as antibiotic) that inhibited the growth of the pathogen, thus presented a clear zone between them. *Aspergillus* also caused abnormal hyphal growth of the pathogen.

Biological control of the disease using *Trichoderma* is a long term process. However, the use of *Trichoderma* is ecologically sound. It is a normal component of fungal flora of the soil with high organic matter content and therefore its natural enemies are already present in the environment (Cuevas *et al*. 2001). *Phoma* and *Trichoderma* isolates gave stable and conspicuous suppressive effect against various soilborne pathogens compared with *Mucor* sp., *Penicillium* spp., and *Fusarium equiseti* isolates (Hyakumachi 2000). The presence of *T. harzianum* in the substrate alongside *P. capsici* generally resulted in a significant decrease in the population density of the pathogen (Ahmed *et al*. 1999). Manohara *et al*. (2003a) reported that *T. harzianum* could be formulated into substrates and pellet. The substrate containing the mixture of soils and coarse grass *(Imperata cylindrica)* was better for sporulation than other substrates, while the formulation of pellet containing the least rice bran and soil showed the highest sporulation. The formulations of substrate and pellet reduced foot rot disease intensity about 66.7% and 36%, respectively.

Recognizing the hazard of pesticides to man and his environment, many countries in the world today are considering biological control as a better alternative to chemical control of plant diseases. The utilization of microorganisms as biocontrol agents has been considered for a long time. Recently, greater efforts are directed toward research and development of biocontrol application techniques. The results of the present study showed that all of fungal colonies isolated from black pepper rhizosphere have the

ability to inhibit the growth of *P. capsici in vitro*. It also showed that fungal population (saprophytic fungi) is positively correlated with the organic matter content in the soil. The addition of organic amendment to soil promotes growth of saprophytic fungi which in turn reduces the populations of *P. capsici*. This report suggests that compost can be used as a source of organic matter because according to Hoitink and Boehm (1999), compost will provide (a) successful competition for nutrients by beneficial mycoflora, (b) antibiotic production by beneficial mycoflora, (c) successful parasitism against pathogens by beneficial mycoflora, and (d) activation of disease resistant genes in plants by mycoflora (induced systemic resistance). The coarse grass and corn *(Zea mays)* amendment increased the population of *T. harzianum* and controlled the foot rot disease incidence on black pepper seedlings (Manohara *et al*. 2003b). Applying the coarse grass to the soil every 3-5 weeks could maintain *Trichoderma* population in the soil with 40% field capacity (Manohara *et al*. 2004). However, the role of mycoflora antagonism in contributing to the activation of disease resistance genes in black pepper plant has not been experimentally established.

CONCLUSION

One hundred and forty nine colonies of soil mycoflora were isolated belonging to fourteen genera; three of them, i.e. *Penicillium*, *Paecilomyces* and *Aspergillus*, are the most dominant. All of the mycoflora isolates are able to inhibit the growth of the pathogen *(Phytophthora capsici)*, eighteen of them are the most promising antagonists based on their inhibition growth with more than 60%.

The organic matter percentage in the soil is significantly correlated with the number of antagonistic mycoflora in the soil, but the cropping pattern is negatively correlated. Results of the present study suggests that organic matter increased antagonistic mycoflora in black pepper rhizosphere, which will reduce severity of the disease.

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